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Anti-idiotypic antibodies (antibodies directed at the combining site of an antibody molecule) are being tested in a new method for safe and efficient vaccination to substances which are too toxic for direct inoculation. Exotoxin A, an extracellular toxin produced by Pseudomonas aeruginosa is being used as the model toxic substance. A panel of monoclonal mouse antibodies to exotoxin A were prepared in NMRI and Balb/c mice, consisting of one IgG1, one IgA and six IgM hybridoma cell lines. Control monoclonals

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20. Abstract

were developed from an existing panel of anti-rickettsia monoclonals of NMRI allotype. The monoclonals were purified by preparative centrifugation and column chromatography and were used to prepare anti-idiotypic antisera in mice and rabbits. High-titered anti-idiotypic antisera to one IgM anti-exotoxin monoclonal, TC-31, was raised in C57B1/6 mice. The antiserum was specific for TC-31 and noncross-reactive with the other monoclonals. High-titered anti-idiotypic antisera to each monoclonal have been prepared in rabbits. The rabbit anti-idiotypic antisera reveal minor antigenic cross-reactions between the anti-exotoxin monoclonals. Rabbit anti-TC-31 idiotypic antibody has been purified from the antiserum and used to develop an assay for the TC-31 idiotype in immune sera from mice immunized with the toxoid of exotoxin A. Thirteen different strains of mice have been immunized successfully with the toxoid. Peak antibody production is reached by day 21 after three weekly inoculations of toxoid in Freund's complete adjuvant. The in vivo mouse model will be used to test the prepared anti-idiotypic antibodies for their ability to immunize mice to exotoxin A. An in vitro assay has also been developed for a proliferative response by toxoid-immune mouse spleen cells stimulated in tissue culture with exotoxin A or the toxoid. The assay will be used to test the effectiveness of the prepared anti-idiotypic antisera.

I. Description of Problem and Approach

The goal of the project was to investigate the feasibility of using an anti-idiotypic antibody as a vaccine for toxins or toxic substances. Anti-idiotypic antibodies are antibodies directed at the antigen combining site of an antibody molecule. The "network hypothesis" formulated by Niels Jerne, proposed that the immune system is composed of a network of interacting antibodies and anti-idiotypic antibodies, on lymphocytes and in the plasma. Anti-idiotypic antibodies were proposed to be active in regulating immune responses. Subsequently, it was suggested by A. Nisonoff and others, that anti-idiotypic antibodies could induce antibody production, and therefore be used as a vaccine. Recently, several investigators have been able to show that anti-idiotypic antibodies can induce protective immunity in mice to African tryparosomiasis parasites and can induce neutralizing antibodies to hepatitis B virus and to rabies virus.

In this project the model system for inducing immunity to a toxic substance has been developed using a toxin, exotoxin A (ET), produced by Pseudomonas aeruginosa. The ET system was chosen because of the extensive studies by Dr. O. Pavlovskis and others in the Infectious Diseases Program Center, NMRI, on the role of ET in the pathogenesis of pseudomonas infection in mice. Standard protocols exist for the purification of ET from culture supernatants of P. aeruginosa. The ET used in this project has been supplied by Dr. S. H. Leppla, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick. The toxin has successfully been converted to a toxoid using Dr. Leppla's gluteraldehyde procedure. The toxoid has been used to establish dose

response curves, kinetics of response, and mouse strain variations in the in vivo response to ET as described below.

The proposed goal of the project was to prepare anti-idiotypic antibodies to monoclonal anti-ET antibodies and to test their effectiveness in inducing protective immunity in mice for exotoxin A.

II. Description of the Monoclonal Anti-ET Antibodies

Monoclonal anti-ET antibodies were derived from hybridomas developed by Dr. Darrell Galloway, Pseudomonas Branch, IDPC, NMRI. The anti-ET antibody producing hybridomas were initially produced by fusion of spleen cells from ET immune NMRI mice with Balb/c SP2/0 cells. The hybridomas were detected in the fusion mixture by assay of culture supernatants for anti-ET binding activity in an ELISA assay. The NMRI Balb/c monoclonals consist of four IgM, two IgA, and one IgG1 producing clone. In March 1984, Dr. Galloway prepared five Balb/c x Balb/c IgM producing monoclonals for use in the project. All of the anti-ET monoclonals are listed in Table 1, and designated with a TC number. Only recently, it was revealed that several of the lines listed in Table 1 were derived from the same fusion well during their development and thus, are identical twin clones. The twin clones have been identified as TC-9 and TC-13, TC-37 and TC-39, TC-52.6 and TC53.6, TC-98 and TC-99. Until now, however, all clones had been used in assays as if they were separate entities.

A second group of hybridoma antibodies, also derived from the NMRI mouse strain, with specificity for the species specific protein antigens of Rickettsia typhi and Rickettsia prowazekii (R-SPA) were obtained from Dr. Gregory Dasch, Rickettsia Branch, Infectious Diseases Program

Table 1

Anti-Exotoxin A and Anti-Rickettsial Protein Monoclonal Antibodies Used in This Study

Hybridoma	Isotype	Strain of Origin	Antigenic Specificity
TC-1	IgG,K	NMRI	Exotoxin A
TC-9	IgA,K	NMRI	Exotoxin A
TC-13	IgA,K	NMRI	Exotoxin A
TC-15	IgM,K	NMRI	Exotoxin A
TC-31	IgM,K	NMRI	Exotoxin A
TC-37	IgM,K	NMRI	Exotoxin A
TC-39	IgM,K	NMRI	Exotoxin A
TC-52.6	IgM,K	BALB/c	Exotoxin A
TC-53.6	IgM,K	BALB/c	Exotoxin A
TC-64	IgM,K	BALB/c	Exotoxin A
TC-98	IgM,K	BALB/c	Exotoxin A
TC-99	IgM,K	BALB/c	Exotoxin A
#9	IgA,K	NMRI	SpA
#40	IgA,K	NMRI	TTOT
#67	IgA,K	NMRI	PTOT
#11	IgM,K	NMRI	PTOT
#22	IgM,K	NMRI	PTOT
#33	IgM,K	NMRI	PTOT
#45	IgM,K	NMRI	TTOT

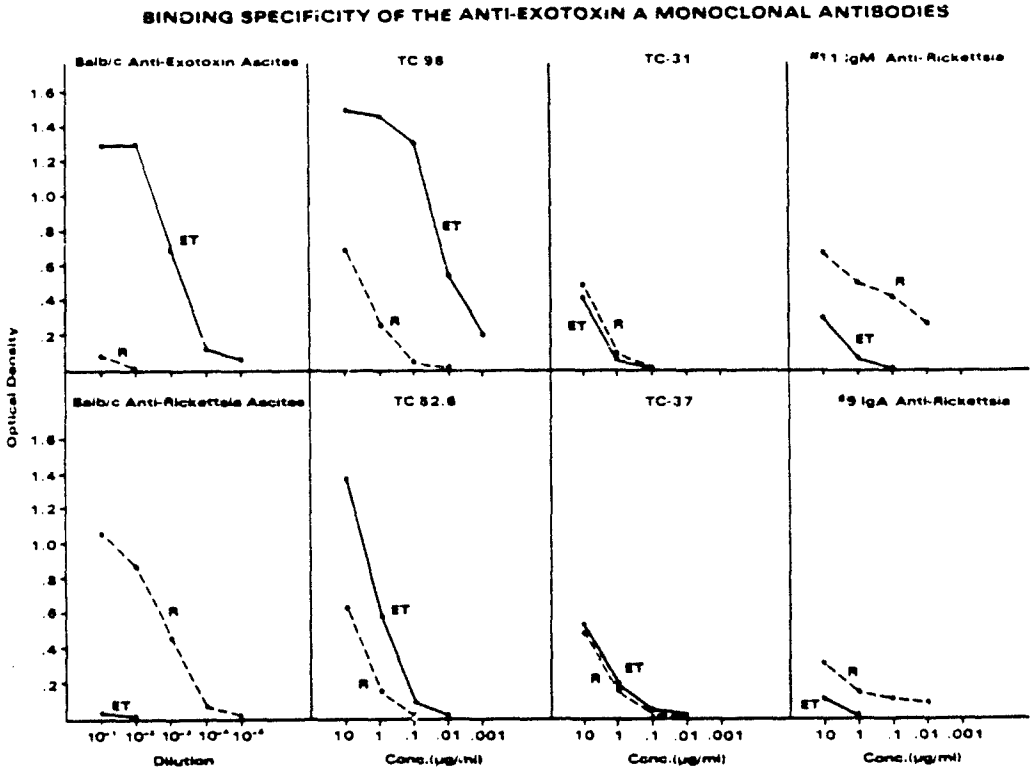
Center, NMRI. These were received as mixed cultures, from which the clones listed in Table 1 were derived by limiting dilution cloning. The clones were typed for antibody isotype and binding affinity for the SPA of Rickettsia prowazekii (PTOT) or Rickettsia typhi (TTOT) in an ELISA

assay. This latter group of hybridomas were chosen for this study to be used as control antibodies in development of anti-idiotypic antisera. Since these antibodies were also derived from the NMRI mouse stain, they carry the same allotypic specificities as the anti-exotoxin monoclones, and are being used as affinity absorbants to remove any anti-allotypic antibodies from anti-idiotypic antisera prepared against the anti-exotoxin hybridomas.

All of the hybridoma lines were grown in sublethally irradiated, pristane-primed Balb/c mice for production of ascites. The monoclonal IgM antibody was purified from the ascites by ultracentrifugation followed by column chromatography on Sepharose 6B. The purity of the monoclonals was accessed by immunoelectrophoresis and they were found only to have alpha$_2$-macroglobulin as a major contaminant in the preparations. The monoclonal IgA's were purified by salt fractionation followed by sepharose 6B column chromatography. The IgG1 was purified on DEAE-Affigel Blue.

The binding specificity of the monoclonals was determined by an ELISA assay, using plates coated with either ET or rickettsial protein (RP). Figure 1 shows the binding specificity of four of the anti ET monoclonals and two anti-rickettsia monoclonals, as well as control ascitic from mice hyperimmune to either ET or P-SPA. The monoclonal anti-ET antibodies bound to ET with varying degrees of specificity. Monoclonals TC-31 and TC-37 were found to completely cross-react with RP, while TC-98 was very specific for ET. The IgG1 monoclonal anti-ET, TC-1 also showed only minor cross-reactivity on RP (data not shown) with an OD of 0.984 on ET at 7 μg/ml and 0.041 on RP at 700 μg/ml.

Figure 1



An important aspect of this project was to determine which of the hybridomas were able to neutralize the toxic effects of ET. Anti-idiotypic antibodies to these monoclonals would be used to immunize mice for a lethal challenge of ET. The most reactive hybridomas were tested for their ability to protect mice from a lethal challenge with ET. A preliminary experiment showed the LD_{50} of the ET being used in this project as being 0.3 µg. To test the monoclonal anti-ET antibodies, mixtures of ET and various amounts of purified monoclonal antibody were incubated for 2 hr at 37°C and then injected into mice IV. The mice received 1 µg ET or 3 LD_{50} doses. The survival of the mice was

monitored over the next 72 hr. Control animals receiving ET alone die within 48 hr. The results of several experiments are shown in Table 2. None of the hybridomas which were tested were able to protect the mice, however, control animals given as little as 100 μg of a hyperimmune globulin preparation from toxoid immunized mice, was able to prevent death. Studies are still in progress to determine if any of the other hybridomas are protective alone or in combinations.

Table 2

Studies on Protection In Vivo with Monoclonal Anti-Exotoxin Antibodies

Group	Hybridoma	(μg) Dose	(1 μg) Exotoxin	No. Mice Live/Total
Exp. 1:				
1a	TC-98	500	-	5/5
1b	TC-98	500	+	0/5
2a	TC-52.6	500	-	5/5
2b	TC-52.6	500	+	0/5
3a	TC-31	500	-	5/5
3b	TC-31	500	+	0/5
4a	Hyperimmune glob.	1000	-	5/5
4b	" "	1000	+	5/5
5	-	-	+	0/5
Exp. 2:				
1a	TC-1	500	-	0/5
1b	TC-1	500	+	0/5
2a	TC-98	500	-	0/5
2b	TC-98	500	+	0/5
3a	TC-1 + TC-98	500 + 500	-	0/5
3b	TC-1 + TC-98	500 + 500	+	0/5
4	Hyperimmune glob.	500	+	5/5
5	-	-	+	0/5
Exp. 3:				
1a	TC-1	100	+	0/5
1b	"	10	+	0/5
1c	"	1	+	0/5
1d	"	0.1	+	0/5
2a	Hyperimmune glob.	100	+	5/5
2b	" "	10	+	0/5
2c	" "	1	+	0/5
2d	" "	0.1	+	0/5
3	-	-	+	0/5

III. Production of Anti-idiotypic Antibodies

Mouse anti-idiotypic antibocy: In choosing an appropriate strain of mouse to prepare the anti-idiotype antibodies, it is important to consider the allotype of the monoclonal antibodies being used. Previous studies by Lieberman (PNAS 68:2508, 1971) have shown that the immune response to Balb/c derived IgA myeloma proteins in various strains of mice is dependent upon differences in the immunoglobulin allotype as well as the H-2 type of the recipient strains. It was also demonstrated that, if the allotype of the immunizing protein was the same as that of the recipient strain, no anti-idiotype antibodies were produced, even in recipients of different H-2 type than Balb/c. These data suggest that the allotype determinants were acting as carrier determinants for the recognition of idiotype specifically by B cells.

The allotype of the NMRI mouse monoclonal anti-exotoxin antibodies being used in this study was not known at the start of this project, and this presented a dilemma in terms of choosing an appropriate mouse strain for use in preparing mouse anti-idiotype. Thus, an experiment was designed to test the responsiveness of mouse strains of various H-2 type and allotypes specificities to the NMRI monoclonals. Two IgM anti-exotoxin monoclonals and two IgA anti-exotoxin monoclonals were used to test responsiveness. Groups of 5 mice were immunized with the monoclonals according to the protocol of Lieberman and Potter. Mice received 75 µg of protein in complete Freund's adjuvant on day 1, 75 µg protein in incomplete Freund's adjuvant on day 3, followed by weekly boosts of 75 µg protein in saline for 7 weeks. They were given 6 injections: in the two footpads, and subcutaneously in the axillary and inguinal regions on both sides. The mice were bled from the

retro-orbital sinus weekly beginning on the fourth week of immunization. The mouse antisera were initially tested for presence of anti-idiotypic antibodies in an Ouchterlony analysis against the anti-exotoxin and anti-rickettsia monoclonals. A summary of the results in Table 3 show that it was very difficult to find a strain which would react to the two IgM monoclonals, TC-15 and TC-31. Only DBA/2 responded to both monoclonals, however, the reactions were weak and heterogenous. However, C57B1/6 mice responded strongly to TC-31 with no anti-allotypic reaction detectable. In contrast, a number of strains responded to the IgA monoclonals, TC-9 and TC-13.

TABLE 3

Ouchterlony Analysis of Mouse Anti-sera for Anti-idiotype

	Allotype			Immunogen			
	H-2	IgM	IgA	TC-9	TC-13	TC-15	TC-31
A/J	a	e	d	Id+	Ig+	-	-
AKR/J	k	-	d	-	-	Id+	-
C57B1/6J	b	b	b	-	-	-	Id+++
DBA/2J	d	-	c	Id,Ig+	Ig+	Id,Ig+	Id,Ig+
SJL/J	s	-	b	Id++	Id+++	-	-
C3H/HeJ	k	-	a	-	Id++	-	-
RIII/J	r	-	c	Id+	Id+	-	-
BALB/c	d	a	a	n.d.	n.d.	n.d.	n.d.
NMRI	d/dex	-	a	n.d.	n.d.	n.d.	n.d.

Confirmation of the Ouchterlony patterns was provided by a test for passive hemaglutination of sheep erythrocytes coated with the various hybridoma antibodies. The results of one assay, shown in Table 4,

Table 4

Passive Hemagglutination by Mouse Anti-Idiotypic Antisera

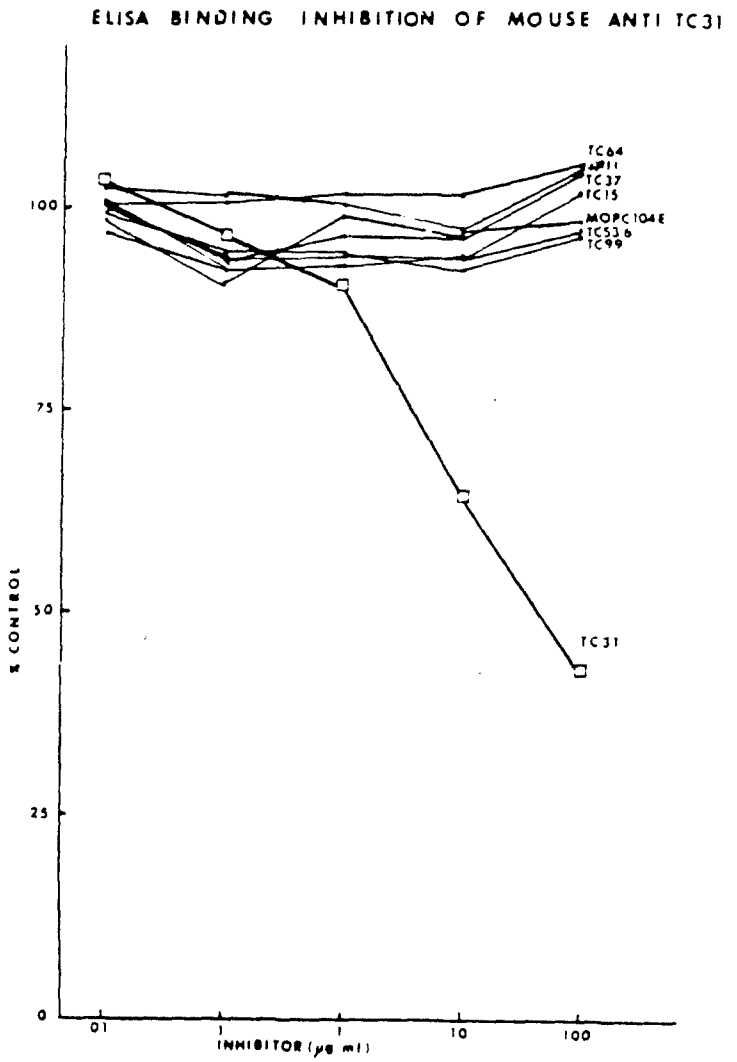
	A,K TC-9	A,K TC-13	M,K TC-15	M,K TC-31	A,K #9	M,K #11
A/J anti-TC 9	1280	1280	0	0	0	0
TC 13	160	160	0	0	0	0
TC 15	0	0	0	0	0	0
TC 31	0	0	0	0	0	0
AKR anti-TC 9	160	160	0	0	0	0
TC 13	0	0	0	0	0	0
TC 15	0	0	160	±10	0	0
TC 31	0	0	0	±10	0	0
C57Bl/6 anti-TC 9	0	0	0	0	0	0
TC 13	40	40	40	40	40	40
TC 15	10	10	80	10	10	±10
TC 31	0	±10	0	320	±10	0
DBA/2 anti-TC 9	2560	2560	0	±10	0	0
TC 13	1280	1280	0	±10	0	0
TC 15	0	40	40	±10	0	0
TC 31	0	0	0	320	0	0
SJL/J anti-TC 9	5120	10,240	0	0	0	0
TC 13	5120	5120	0	10	0	0
TC 15	10	40	20	10	10	10
TC 31	0	0	0	40	0	0
C3H/HeJ anti-TC 9	160	160	0	±10	0	0
TC 13	1280	1280	0	±10	80	0
TC 15	0	0	0	±10	0	0
TC 31	0	0	0	±10	0	0
BALB/c anti-TC 9	2560	5120	0	0	0	0
TC 13	2560	2560	0	0	0	0
TC 15	0	0	0	0	0	0
TC 31	0	0	0	0	0	0

confirm the conclusions mentioned above. C57B1/6 immunized with TC-31 showed a titer of 1:5120 against TC-31 coated SRBC and this anti-serum was essentially non-reactive with the other monoclonals. C57B1/6 immunized with TC-15, only gave a titer of 1:80 against TC15. SJL mice immunized with TC-9 or TC-13 gave titers of 1:5,120 to 10,240 on both monoclonals and these anti-sera did not react with the IgA anti-rickettsia monoclonal.

From these experiments it was decided to choose the C57B1/6 strain for production of mouse anti-TC-31 idiotype and the SJL strain for production of anti TC-9 and TC-13 idiotype. Further experiments are planned to attempt to induce responsiveness in C57B1/6 mice to the other IgM monoclonals by immunizing with conjugates of keyhole limpet hemocyanin.

An ELISA assay was performed to confirm the specificity of binding of the C57B1/6 anti-TC-31 idiotypic antisera. The antiserum was incubated for 2 hrs at 37°C with various concentrations of TC-31 or other anti-ET-monoclonals or anti-RP monoclonals. The mixtures were then plated on ELISA plates coated with TC-31 and assayed for the amount of C57B1/6 anti-TC-31 which could still bind to TC-31 on the plate. Figure 2 shows that only TC-31 was able to inhibit binding of the C57B1/6 anti-serum. No inhibition was seen by five other anti-ET monoclonals or by #11 anti-RP or MOPC104E (Balb/c anti-dextran myeloma protein). These results demonstrate that the antiserum is only reactive with TC-31 idiotype and does not contain anti-immunglobulin (anti-Ig) or anti-allotype antibody.

FIGURE 2



2. Rabbit anti-idiotypic antibody: Rabbits were immunized with 100 ug of purified monoclonal antibodies in complete Freund's adjuvant. The antiserum was tested for anti-idiotypic antibody by an ELISA assay. The antisera were diluted on ELISA plates coated with the immunizing monoclonal or other monoclonals. This was done in the presence of an

excess amount of ascites containing #11 anti-RP monoclonal to block the anti-Ig or anti-allotype antibody in the serum. The antiserum was also plated on an ELISA plate coated with #11 as a control. The results in Table 5 tabulate a series of experiments designed to test for anti-idiotypic antibody and the specificity of the antibody.

TABLE 5

CROSS-REACTIVITY OF RABBIT ANTI-IDIOTYPIC ANTISERA

RABBIT	TC15	TC31	TC37	TC39	TC64	TC98	TC99	TC52.6	TC53.6	TC9	TC13
E41 anti-TC99	-	-	-	-	-	+++	+++	-	-	+	++
E77 anti-TC99	-	-	++	-	-	+++	+++	-	-	+	++
E47 anti-TC64	++	-	-	-	++	-	-	-	-	-	++
E73 anti-TC64	++	++	-	++	+++	-	-	-	-	-	++
E39 anti-TC39	++	-	+++	+++	-	-	-	-	-	++	++
E43 anti-TC39	++	-	+++	+++	-	-	-	-	-	++	++
107D anti-TC31	-	+++	+	+	+	+	-	-	-	+	+
R02 anti-TC31	-	+++	++	++	++	-	-	-	-	-	-
119D anti-TC15	+++	+	++	++	-	++	-	-	-	-	-
123D anti-TC15	++	+	-	-	-	++	-	-	-	-	-
E63 anti-TC37	-	-	+++	+++	-	-	-	-	-	+	+
E71 anti-TC37	+	-	+++	+++	-	-	-	-	-	+	+
E57 anti-TC13	+	+	+	+	-	-	-	-	-	+++	+++
E67 anti-TC13	+	+	+	+	+	-	-	-	-	+++	+++
E55 anti-TC9	+	+	+	+	+	-	-	-	-	+++	+++
E65 anti-TC9	+	+	+	+	+	-	-	-	-	+++	+++
R00 anti-het. Ig	+	+	+	+	+	-	-	-	-	-	+
Q96 anti-het. Ig	-	+	-	-	-	-	-	-	-	-	-

Each monoclonal was used to immunize two rabbits. The antisera was tested for binding to each monoclonal. The results show strong binding of each antisera to its respective immunizing monoclonal. Equally strong binding of the twin monoclonal is seen as in the case of E41 anti-TC99 binding to both TC-99 and TC-98. Minor cross-reactions are

seen with some other monoclonals. R00 and Q96 represent rabbits immunized with hyperimmune anti-ET globulin. These antisera show minor reactions with some monoclonals. Further confirmation of the crossreactives in this Table await purification of the anti-idiotypic anti-sera.

Rabbit 107D anti-TC-31 antiserum was absorbed over affinity columns of various Balb/c myeloma proteins representing whole serum proteins, IgM, IgA, IgG1, 2a, 2b, and IgG3. The ability of this antiserum before and after absorption to agglutinate TC-31 or #11 coated erythrocytes was tested as shown in Table 6. The non-absorbed serum had high titers to TC-31 as well as #11 and MOPC104E, however, absorption over affinity

TABLE 6

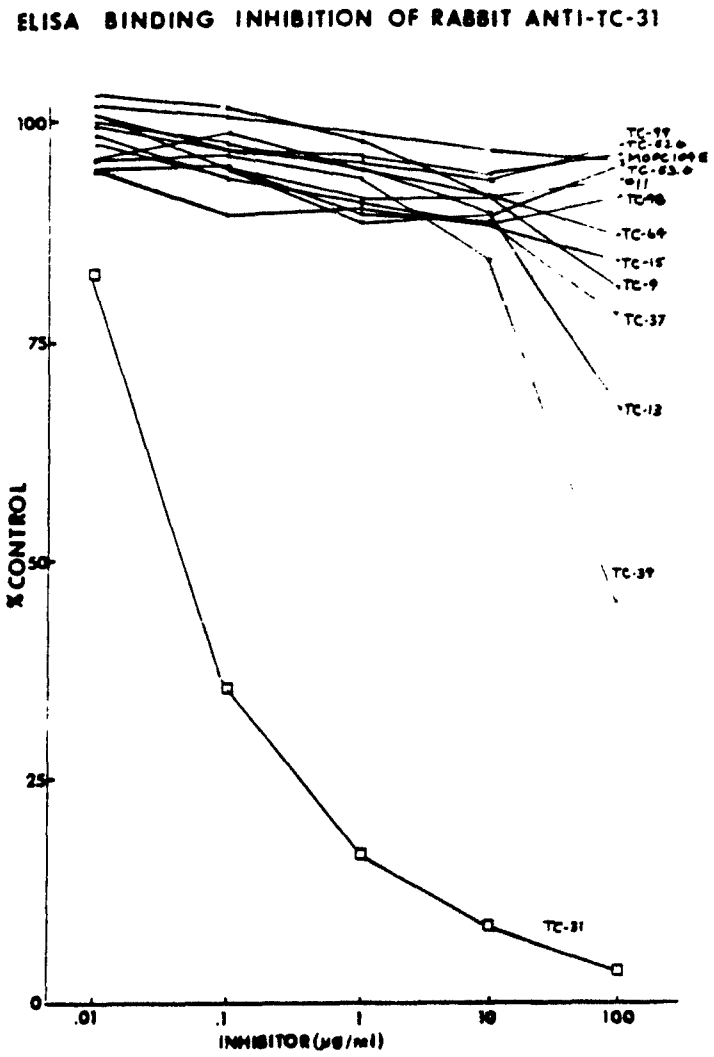
Passive Hemagglutination of Rabbit
Anti - TC - 31 Antiserum

Serum	SRBC Conjugate	Titer
107D	TC-31	5120
107D Abs.	TC-31	1280
C57B1 anti TC 31	TC-31	320
107D	#11	2560
107D Abs.	#11	40
C57B1 anti-TC 31	#11	0
107D	MOPC 104E	1280
107D Abs.	MOPC 104E	0
C57B1 anti-TC 31	MOPC 104E	0

columns removed all of the activity for MOPC104E (anti-dextran) and the majority of activity to #11 (anti-RP), while activity on TC-31 remains high at 1:1280. In contrast, the C57Bl/6 anti-TC-31 serum only agglutinates TC-31 coated erythrocytes.

The same 107D antiserum was further absorbed over an affinity column of #11 anti-RP IgM, and then tested in an ELISA inhibition assay. The absorbed antiserum was incubated 2 hr with various concentrations of

Figure 3



purified monoclonals and then plated on a TC-31 coated ELISA plate. The amount of rabbit anti-TC-31 bound to the plate was detected with an alkaline phosphatase conjugated goat anti-rabbit IgG. As seen in Figure 3, TC-31 completely inhibits the binding of the antiserum, while partial inhibition is seen with TC-39, TC-13, TC-37, and TC-9 at the highest concentrations of inhibitor. These results suggest that 1070 antiserum contains antibiotics which react with a common antigenic determinant on TC-31, TC-39, TC-37, awaits purification of this subset of antibodies in 107D antiserum.

The 107D antiserum was further passed over a Protein A sepharose column to isolate the globulin fraction containing anti-idiotypic activity. This purified anti-idiotypic antibody was used to coat ELISA plates. Antibody on the plate was detected by an alkaline phosphatase - conjugated TC-31 (AP-TC-31) preparation. In the future, this assay system will be used as an inhibition assay to detect TC-31 idiotype positive antibody in mice immunized with anti-idiotypic antibody to induce the TC-31 idiotype positive anti-ET antibodies. To test the assay system, secondary immune antisera from toxoid immunized (day 21 bleed of antisera displayed in Figure 5) mice were used to inhibit the binding of AP-TC-31 to the plate. Table 7 shows the results of this inhibition assay. Several strains of those tested showed inhibition of TC-31 binding. The small amount of inhibition found in all positive strains indicates that the TC-31 idiotype is only carried on a small fraction of anti-ET antibodies and is not a dominant idiotype for the anti-ET immune response.

TABLE 7

Inhibition of TC-31 binding to 107D rabbit anti TC-31 ELISA plate by ET immune sera

Serum from ET immune mice	Antiserum Dilution 1:10	$1:10^2$	$1:10^3$	$1:10^4$	1:105	Presence of TC-31 Idiotype
	% Inhibition					
A/J	0	9.1	1.0	0	0	-
AKR	10.1	2.0	1.8	1.8	0	+
Balb/c	10.7	11.9	5.2	0	0	+
C3H/HeJ	0	0	0	0	0	-
C57B1/6	0	0.8	2.9	0	0	-
CB20	9.5	3.8	3.5	0	0	+
CBA/J	16.2	6.2	0	1.3	0	+
CEJ	3.5	0	3.2	1.3	0	±
DBA/1	13.2	6.7	1.5	0	3.7	+
DBA/2	16.5	11.8	4.1	1.5	0	+
NMRI	17.4	9.0	3.9	2.0	0.6	+
RIII	16.5	12.6	0	0	0.2	+
SJL	0	0	0	1.3	0	-

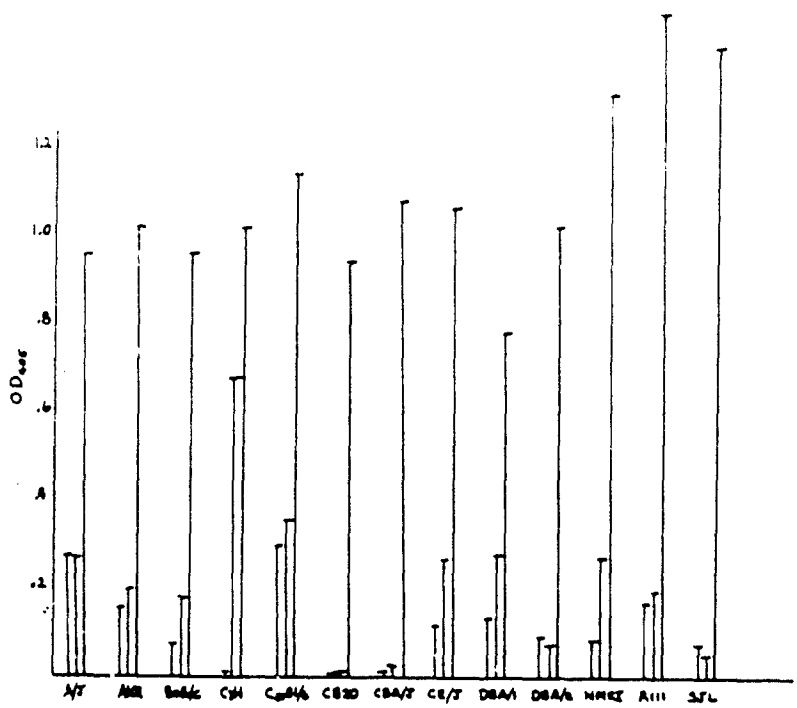
IV. Models for testing the effectiveness of anti-idiotype as a vaccine.

Before immunization of mice with anti-idiotype could be performed,

the characteristics of the response to the toxoid of ET had to be investigated. Various strains of mice were given two injections of toxoid in complete Freund's adjuvant and bled on day 7 and 21. Figure 4 shows the results of an ELISA direct binding assay for anti-ET antibodies in the normal serum and 1° and 2° bleeds from these mice. The mouse antibodies are being detected by a goat anti-mouse IgG + IgM reagent. Although our entire mouse colony is maintained on acidified water and sterile feed to minimize the chances of the mice being infected with Pseudomonas aeruginosa, some strains have high background titers to ET (first bar in each triplet). It is unknown whether or not this represents cross-reactive antibody to other microorganisms in the

FIGURE 4

IN VIVO RESPONSE TO EXOTOXIN A

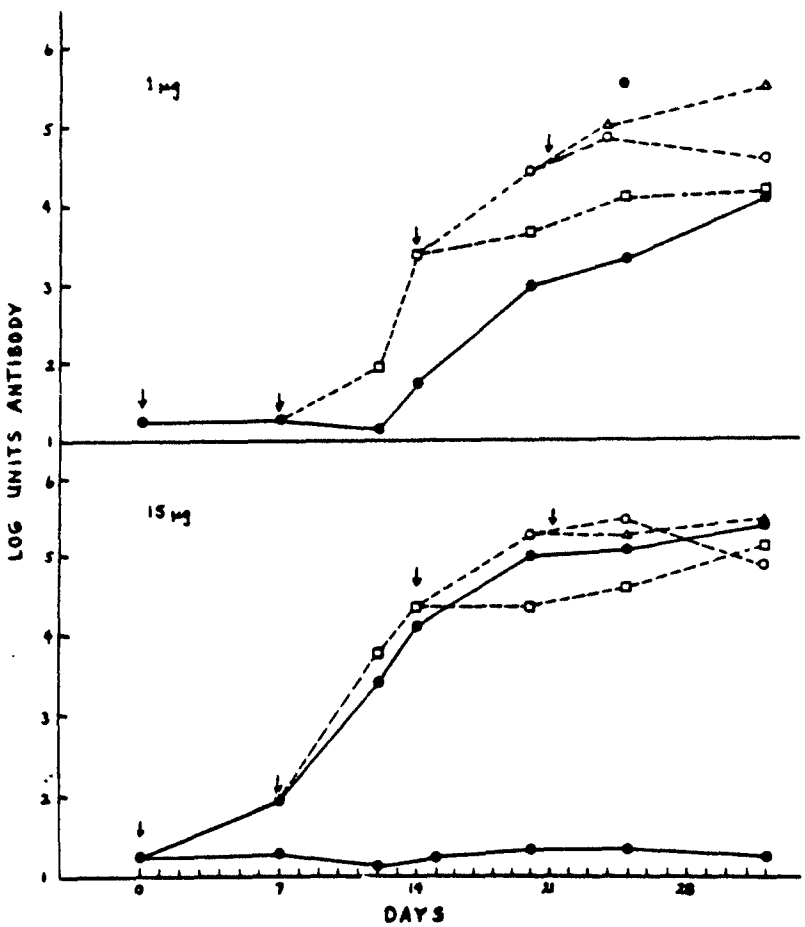


intestinal flora. The response 7 days after the first immunization (2nd bar of each triplet) is very weak, however, after a booster injection on day 10, the day 21 response (3rd bar) is very good in all strains tested.

The lag in responsiveness was further documented in a study of the kinetics of the response to ET in Balb/c mice shown in Figure 5. The arrows represent time of immunization with either 1 or 15 μg of toxoid in complete Freund's adjuvant on day 0, incomplete Freund's adjuvant on day 7, and saline on days 14 and 21. The break in the graph represents

FIGUPE 5

IN VIVO ANTIBODY RESPONSE TO EXOTOXIN A TOXOID



different groups of mice which were given only 1,2,3 or 4 immunizations with toxoid. The antibody binding ET is assayed in an ELISA assay for total immunoglobulin (G+M). A lag in response occurs until day 7 to 10 and the response does not plateau until the third week. The amount of immunogen given does not seem to influence these delayed kinetics of responsiveness.

This in vivo model will be used to assay for the effects of anti-idiotypic antibody administration. The response to toxoid will be compared and contrasted with the response to anti-idiotype alone. The ability of anti-idiotype to substitute for toxoid in the immunization scheme will also be tested.

Anti-idiotype will also be tested in vitro for its effects on an in vitro proliferative response to ET or toxoid in cultures of immune spleen cells, as shown in Figure 6.

The data in Figure 6 represents a day 5 response of spleen cells from mice given two immunizations with toxoid 3 months previously. The peak response occurs around day 5, and thus is most likely a T cell proliferative response, although this must be tested by use of cell separation techniques.

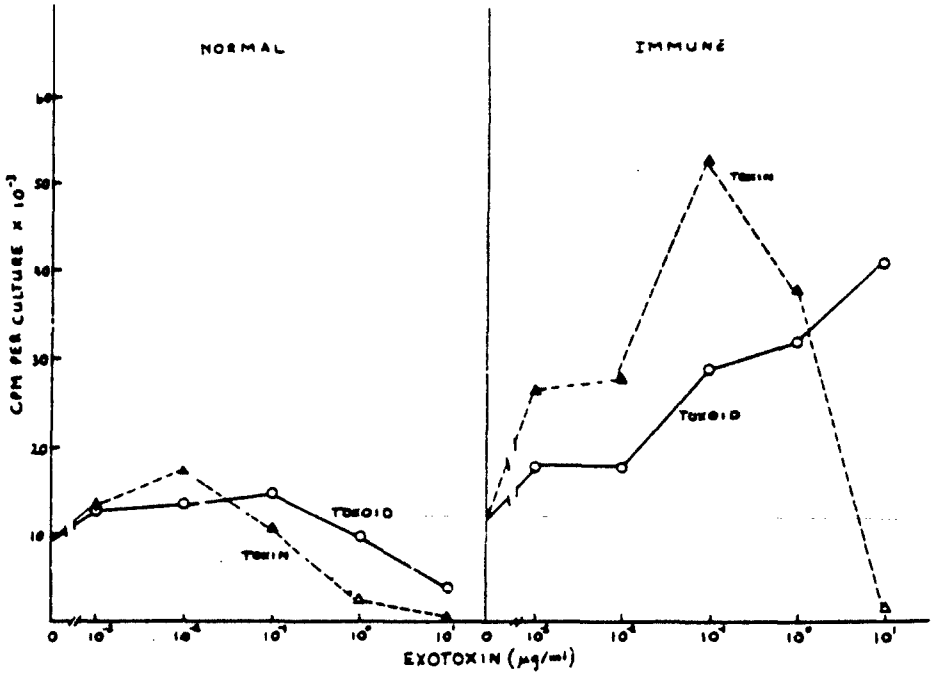
V. Future Research

Continuing efforts on this project will include the following:

1. Development of hybridoma anti-ET antibodies which are protective.
2. Continued purification and testing of anti-idiotypic antibodies in an effort to find a cross-reactive or dominant idiotype for the response to ET.

Figure 6

PROLIFERATIVE RESPONSE TO EXOTOXIN A



3. Testing of anti-idiotypic reagents in vivo for induction of anti-ET antibodies or induction of antibodies carrying the appropriate idiotypic specificity.